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Original Article

PRELIMINARY STUDY ON ALGINATE CONCENTRATION AND ANTIBACTERIAL ACTIVITY OF PALMAROSA ESSENTIAL OIL

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ABSTRACT

Objective: Palmarosa (*Cymbopogon martinii* (Roxb.)) essential oil has volatile active compounds, therefore, it requires modification of encapsulation to obtain optimum potency. This study investigated the relationship between various alginate concentrations in microencapsulation against the quality of the formula and antibacterial activity.

Methods: The study use Palmarosa Essential Oil (PEO) that distillated at Rumah Atsiri, Indonesia. Ionic gelation was used to prepare microencapsulations at different alginate concentrations of 0.5%, 0.75%, and 1.5%. The investigation involved Fourier Transform Infrared (FTIR), organoleptic, morphological, microencapsulated weight, Encapsulation Efficiency (EE), and antibacterial activity.

Results: The organoleptic observation results for all formulas are white in color, have a pronounced PEO scent, and contain spherical particles with macrometer-sized morphology similar to soft beads. The result FTIR showed that F1, F2, and F3 contain aromatic ring, primarily alcohol, alkene, alkyl, and alcohol. The results showed that F1, F2, and F3 were included in the microencapsulation range, namely 5-5,000 µm. Formula III had the greatest EE of 86.53±0.75% and antibacterial activity against *Staphylococcus epidermidis* and *Pseudomonas aeruginosa,* respectively showed inhibition zones with diameters of 12.30±0.16 mm and 7.60±0.24 mm.

Conclusion: This study revealed that the findings of this study demonstrate that the concentration of alginate in microencapsulation influences the properties and antibacterial activity of PEO. Higher alginate concentrations can lead to increased EE, particle size distribution, and ultimately leading to enhanced antibacterial activity.

Keywords: Alginate, *Cymbopogon martini,* Ionic gelation, Microencapsulation, Palmarosa

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INTRODUCTION

Palmarosa (*Cymbopogon martinii*) essential oil has volatile active compound such as geraniol (83,75%), geranyl acetate (9.82%), linalool (1.55%), trans caryophyllene (1.17%), and geranyl hexanoate (0.99%) [1]. The formation of encapsulation could increase the stability of essential oils by protecting the essential oils from the effects of temperature, light, and oxygen, so that the essential oils not easily evaporate and easily oxidized. The stability of encapsulated eucalyptus essential oil could protect its chemical and physical properties for one year at room temperature [2].

Encapsulation is the process of including one substance in another substance. The trapped substance is called the active substance. The substance that is outside or traps the active substance is called the matrix. The choice of matrix is influenced by the physicochemical properties of the active substance, the concentration of the active substance, the requirements for release properties, the final use of the active substance, and the final product. The stability of the ionic gelation method could be obtained by the interaction between the polymer and the oppositely charged polymer or the polymer with polyanions or polycations [3].

Microencapsulation is a technology for protecting active substances that have micro-sized particles with a diameter of 1–1000 µm. Several microencapsulation methods have been used, ionic gelation is one of them [4]. The development of this type of encapsulation technology is widely applied, especially for antimicrobials. Ionic gelation has many advantages including high Encapsulation Efficiency (EE), scalability, reproducibility, and the use of ambient temperature conditions [5]. Using room temperature is better because the active substance is essential oil. Although ionic gelation has a higher EE compared to other encapsulation techniques, some factors could influence essential oil encapsulation, there as the concentration of essential oil, matrix, and cross-linking compounds [6].

Microencapsulation of essential oils is a promising technique for the cosmetic industry, offering protection and controlled release of these active agents. The antimicrobial and antioxidant properties of essential oils make them valuable in cosmetic applications, and their microencapsulation can further enhance these properties [7]. In addition, some essential oil constituents contain unstable functional groups and can change depending on conditions, such as pH and degree of hydration, its changes can affect the antimicrobial properties of the essential oil. Therefore, the encapsulation of essential oils in a suitable carrier material could help improve their stability, water solubility, and controlled release, which enhance their antibacterial activity [8].

Therefore, this study aimed to develop a microencapsulation formulation with variation of alginate concentration, which demonstrated antibacterial activity. Encapsulation of Palmarosa Essential Oil (PEO) was expected to improve the antibacterial effect. Evaluation of physical characterization for microencapsulation produced was also carried out to evaluate the quality of the microencapsulation of PEO.

MATERIALS AND METHODS

Chemicals and biologicals

PEO (Rumah Atsiri, Tawangmangu, Indonesia), $CaCl_2$ (Brataco Chemical), Sodium alginate (Brataco Chemical), tragacanth (Brataco Chemical), Aquadest, 96% ethanol pro analyses (Merck, Germany), Mueller Hinton (MH) agar (Oxoid, UK), Nutrient Broth (NB) (Oxoid, UK), and *Staphylococcus epidermidis* (Merck), *Pseudomonas aeruginosa* (Microbiologics).

Equipment

The equipment used were mortar, 50 ml volumetric flask, test tube, microbial loop, paper disc, pH meter, Fourier Transform Infrared (FTIR) spectrophotometer (PerkinElmer), Rotary evaporator (Buchi R-300, USA), Analytical balance (Ohaus Adventurer AR2140, UK), magnetic stirrer, Incubator (Nesco DSI-500D, Taiwan), UV-Vis spectrophotometer (Shimadzu).

Microencapsulation preparation

In this study, variations of sodium alginate were applied to the formulation of PEO microcapsules. This formula was based on the results of optimization previously conducted [9, 10], including the preparation of PEO emulsion and the fabrication of PEO microcapsules, 10 g of the PEO microcapsule formulation being made [10]. Data on the PEO microcapsule formula can be seen in table 1.

Table 1: Formula used for the microencapsulation formulations

Composition	Formula (% b/v)		
	F1	F2	F3
PEO	30	30	30
Tragacanth	0.5	0.5	0.5
Sodium alginate	0.5	0.75	1.5
Aquadest	Add 100	Add 100	Add 100

Sodium alginate and tragacanth were weighed based on table 1; then it was dissolved with aquadest. Then, it homogenized using a stirrer at 1000 rpm. After that, PEO was gradually added to the mixture and homogenized until the emulsion was formed. The microencapsulation process was carried out using the ionic gelation method, where the prepared homogenous mixture was placed into a syringe with a 30 G needle, and then the mixture was dropped into a 0.5 mol/l $CaCl_2$ solution to form microcapsules. After the microcapsules were formed, the beads were left to stand for 20 min before being filtered [9].

FTIR spectrum analysis

10 mg of sample was loaded onto the diamond crystal of the ATR FTIR spectrometer. The background was recorded with a clean crystal before the start of the measurement and before each new sample. FTIR spectra were recorded at scan rates between 4000 and 600 cm^1.

Evaluation of PEO microcapsule

Organoleptic observation of microcapsules was conducted by making direct observations of the shape, color, taste, and smell of microcapsules. The diameter of the formed and filtered PEO microcapsules needs to be determined. Twenty microcapsules from each formula are randomly selected, and their diameters are measured using a digimatic micrometer screw.

Determination of PEO microcapsule yield

The yield of the sample was determined by comparing the total weight of the obtained microcapsules to the weight of the microcapsule-forming materials. Each sodium alginate, $CaCl_2$, PEO, tragacanth was weighed and carefully recorded as the weight of the microcapsule-forming materials, then the results of the microcapsule beads were weighed and recorded as the total weight of the obtained microcapsules, then the data processed to the equation.

Analysis of EE

The amount of PEO absorbed in the microcapsules was determined directly by calculating the total content against the theoretical content added to the formula. About 100 mg of microcapsules were ground in ethanol using a mortar, then transferred into a 50 ml flask and ethanol was added up to the mark. The solution was then diluted to 300 mg/l and its absorbance was measured using a UV spectrophotometer at the predetermined PEO absorption wavelength and then the absorbance data was calculated using a linear regression equation from the PEO calibration curve in ethanol, with the experiment being repeated. The PEO content and EE are then calculated [9, 10].

Bacterial test preparation

The prepared Nutrient Agar medium was poured into a test tube and then tilted; after the nutrient agar solidified, each colony of *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* bacteria was taken using a microbial loop and then streaked onto the surface of the nutrient agar medium and incubated at 37 °C for 24 h. After obtaining pure culture results, one loop of bacteria that had been rejuvenated on nutrient agar media was taken, then suspended in a tube containing 5 ml of nutrient broth media and incubated for 24 h at 37 °C. A paper disc was used on the agar plate, serving as a holder for the antimicrobial substance. The paper disc containing the antimicrobial substance was placed on the agar plate that had been inoculated with the test microbe, then incubated at a specific time and temperature at 37 °C for 24 h [11].

RESULTS AND DISCUSSION

The primary objective of this study is to investigate the effect of alginate concentration on the encapsulation of PEO and its subsequent antibacterial activity. Alginate, a natural polysaccharide derived from seaweed [12], is known for its biocompatibility, biodegradability, and ability to form gel-like structures when in the presence of calcium ions. Sodium alginate could be used as a thickener, suspending agent, stabilizer, and gel [13]. Calcium ion-dependent gelation is important in the use of alginates. The gelation mechanism that occurs between alginate and calcium ions is the classic egg box model [9], which describes two antiparallel chains that form egg box dimers with Ca^{2+} and then aggregate laterally to form multimers. Chelate complex reaction between sodium alginate and calcium chloride [14].

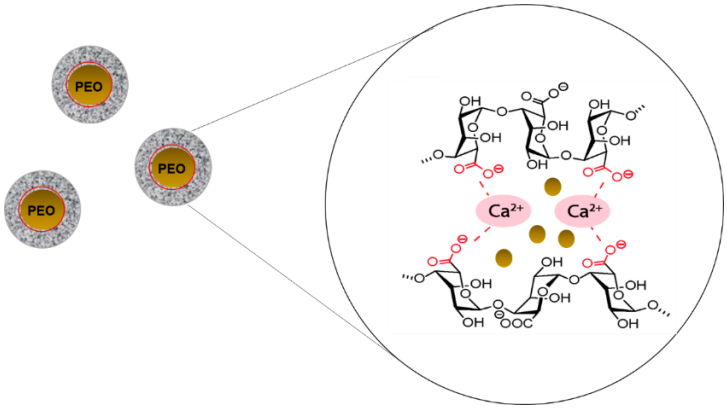

Fig. 1: Ilustration Egg-box model for the interaction of alginate with divalent cations

The preliminary study on the encapsulation of PEO in alginate has provided valuable insights into the potential application of this approach for enhancing the antibacterial activity of the essential oil [15]. To achieve the stated objectives, different concentrations of alginate would be used for PEO microencapsulation using the ionic gelation method. Furthermore, the antibacterial activity of the encapsulated essential oil against specific bacterial strains would be evaluated using well-established methods such as the disc diffusion assay and minimum inhibitory concentration determination.

Profile infra-red PEO microencapsulation

The functional groups in the microencapsulation were predicted using FTIR. This analysis confirms the success of encapsulation; the bonds were determined by interpreting the infrared absorption spectra. Fig. 2 shows the FTIR spectrum of PEO microencapsulation and its constituent ingredients. The strong instance peaks were identified at 3364 cm-1 and 3382 cm-1which indicated the vibration of O-H stretching. In wavenumber 3600 cm-1 there was any hydroxide functional group that came from PEO, then in wave number 1622 cm-1is the alkene functional group and around 1024 cm-1is carbonyl functional group. In addition, the absorption peak of PEO at 1622 cm-1 indicated that the composition of PEO contained small molecules of aromatic hydrocarbons such as aldehydes, phenols, and ketones.

The major absorbance bands present in the spectrum of tragacanth were at 3901, 2928, 1734, 1429, 1320, 1148, 1075, and 1001 cm−1, respectively. The major absorbance bands present in the spectrum of natrium alginate were at 3250, 2323, 2161, 1979, 1595, 1406, 1296, 1024, 948 cm−1. In addition, aromatic ring, primarily alcohol, alkene, alkyl, and alcohol in table 2 was observed in F1, F2, and F3. This confirms that all of formula has a functional group from PEO, tragacanth, and natrium alginate.

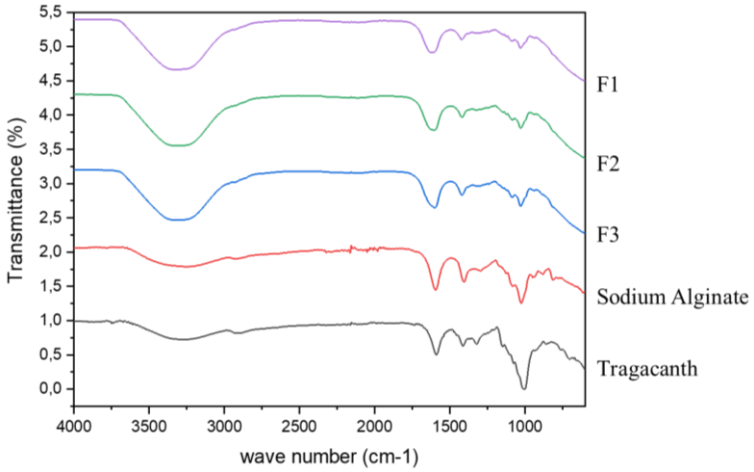

Fig. 2: Infrared spectrum of PEO microencapsulation and its constituent ingredients

Table 2: FTIR spectra peak values and functional groups of PEO microencapsulation and its constituent ingredients

Functional group	Band (cm^1)	Type of vibration
Aromatic ring	714	C-H out of plane bend
Primarily alcohol	1024	C-O strech
Alkene	1622	C=C strech
Alkyl	2927	C-H stretch
Alcohol, hydroxy group	3326	O-H strech

Encapsulation could serve as a promising strategy to overcome the limitations associated with the use of essential oils as antibacterial agents. The controlled release and enhanced stability achieved through alginate encapsulation have the potential to broaden the practical applications of PEO in various antimicrobial formulations and products [16]. Further optimization of the encapsulation process and comprehensive evaluation of the encapsulated essential oil performance in diverse applications. Additional studies focusing on the long-term stability, shelf-life, and compatibility of the encapsulated essential oil with different delivery systems would provide valuable insights for its eventual commercial utilization [17].

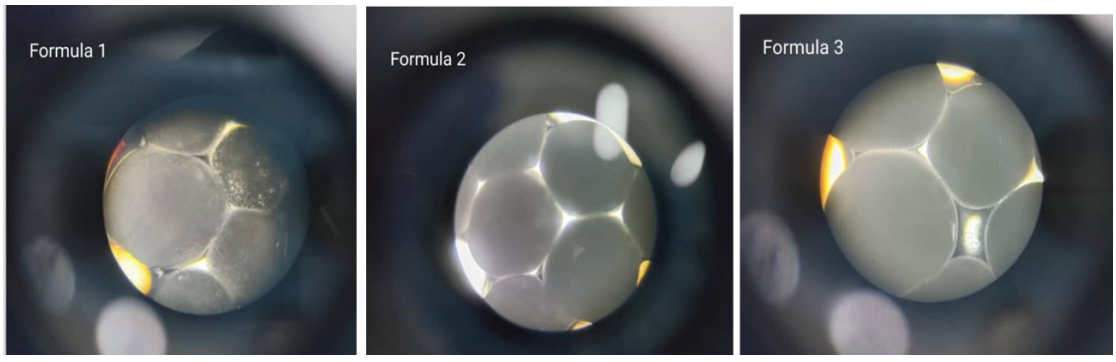

Fig. 3: The form of microencapsulation in light microscopic analysis of each formula (magnified into 40 times)

The organoleptic observation results for all formulas are white in color, have a pronounced PEO scent, and contain spherical particles with macrometer-sized morphology similar to soft beads. The comparison of essential oils, coatings, and crosslinking agents has a significant influence on the percentage of microencapsulation produced. The result in table 3 showed that the formulation with a sodium alginate concentration of 1.5% had a higher value than the concentration of sodium alginate 0.5% and 0.75%.

Table 3: Recovery, diameter encapsulation, and EE of microencapsulation of PEO

Formula	PEO: Tragacanth: sodium alginate	Recovery of encapsulation (%)	Diameter of encapsulation (µm)	EE (%)
F1	30:0.5:0.5	72.0±1.6	33.5±0.19	71.31±0.80
F2	30:0.5:0.75	77.9±1.3	33.78±0.59	78.32±0.38
F3	30:0.5:1.5	84.5±2.6	36.14±0.98	86.53±0.75

Data were presented in mean±SD, n=3

Concentration of 1.5% sodium alginate was able to increase the recovery encapsulation to 84.5±2.6%; this could be caused by increasing the concentration of sodium alginate as a polymer which increasingly forms more bonding between the carboxylate groups of alginate and Ca^{2+}ions [18], it made PEO was encapsulated more optimal.

Measurement of the diameter of PEO microencapsulation was carried out using measurements from a light microscope. Particle size distribution was a physical evaluation of microencapsulation for determining the average diameter of a particle. The particle size distribution of each formula can be seen in table 3. The diameter distribution from each formula was not uniform. The differences are influenced by the ratio of the amount of polymer used. The larger the ratio alginate, the larger form of the particle size [19]. The various sizes of microencapsulation were influenced by several factors, including polymer concentration, the distance between the syringe and the solution when dropping the emulsion into $CaCl_2$, and the pressure difference when forming microcapsules through a syringe. The larger the syringe number used, the smaller the size of the microcapsules that would be produced. The shape of the microcapsules could be formed homogeneously if an instrument such as a peristaltic pump was used, making it easier to carry out the encapsulation process using this method [20].

EE is an important factor to determine whether the method used is appropriate to protect the active substance. EE is determined by comparing the weight of the essential oil in the microencapsulation to the total weight of the essential oil used in making the microencapsulation [21]. The microcapsules formed were compared with the total ingredients forming the microencapsulation, including sodium alginate, tragacanth, aquadest, and PEO.

The formulation with the highest EE value is the F3, namely 86.53±0.75%. The results of this EE analysis showed that the ratio of PEO and matrix in the encapsulation process has influenced the EE. Sodium alginate polymer with a greater concentration in the encapsulation can increase EE because the chance of PEO not being encapsulated is smaller. EE is also influenced by the concentration of the crosslinking agent used [22], but in this study the crosslinking agents were made the same. The essential oil used before the emulsification process is expected to evaporate so that the amount is reduced, this is also a factor causing the decrease in PEO levels in the encapsulation.

The increase in EE with higher alginate concentrations suggests that a higher amount of alginate promotes better entrapment of the PEO within the gel-like structures, leading to improved stability and controlled release [23]. Moreover, the particle size distribution analysis demonstrated that higher alginate concentrations resulted in smaller and more uniform particles, that is advantageous for achieving a more predictable and sustained release of the essential oil. This controlled release behavior is particularly important for optimizing the antibacterial efficacy of the essential oil, as it ensures a prolonged exposure of the bacterial strains to the active constituents.

In terms of antibacterial activity, the PEO microencapsulated exhibited enhanced inhibitory effects against the tested bacterial strains compared to the non-encapsulated oil. This enhancement is attributed to the improved stability and sustained release provided by the alginate encapsulation. The mechanism for inhibiting bacteria by PEO is by damaging the cell membrane, resulting in cells experiencing leaks and changes in morphology. Apart from that, the presence of PEO can cause the release of Ca^{2+} and K+ ions from bacteria. The antimicrobial activity of essential oils is caused by the presence of terpenoid compounds. The water phase in bacterial cells is replaced by terpenoids which are lipophilic, resulting in membrane expansion, increased fluidity and permeability, protein disruption, respiratory inhibition, and changes in ion transport processes [24].

Staphylococcus epidermidis is a type of Gram-positive bacteria, while *Pseudomonas aeruginosa* is a Gram-negative bacteria. Based on table 4, it can be seen that the inhibitory activity of microencapsulation on Gram-positive bacteria is greater than on Gram-negative bacteria. Gram-positive and negative bacteria have a cytoplasmic membrane surrounded by a cell wall. Between these two layers is the periplasmic space which contains various ions [25] and proteins required for various functions involving cellular (electron) transport, substrate hydrolysis, degradation, and detoxification [26]. In g-negative bacteria, the periplasm occupies the space between the plasma membrane and the outer membrane [27]. The presence of an outer membrane in Gram-negative bacteria adjacent to the periplasmic space is the main difference from Gram-positive bacteria, because it is absent in Gram-positive bacteria [28]. The outer membrane consists of a lipid bilayer, where the inner part consists of phospholipids and the outer part lipopolysaccharide [29]. The cell wall of Gram-positive bacteria is composed of many layers of peptidoglycan around 30-100 nm [30], while the cell wall of Gram-negative bacteria is 2-7 nm [31]. The cell walls of Gram-negative bacteria contain more lipids, making them more difficult for essential oils to penetrate [32]. The cell walls of Gram-positive bacteria contain small amounts of lipids and peptidoglycan which are composed of several peptides linked together by polysaccharides [33]. The outer membrane of Gram-negative bacteria is close to the periplasmic space [34], so the membrane would be better protected from environmental influences due to the detoxification function of the periplasm, which causes Gram-negative bacteria to be more resistant to antibacterial compounds [35].

Formula 3 had the greatest antibacterial activity against *Staphylococcus epidermidis* showing inhibition zones in table 4 with diameters of 12.30±0.16 mm and *Pseudomonas aeruginosa* with diameters of 7.60±0.24 mm. The enhanced inhibitory effects of the encapsulated PEO against the bacterial strains compared to the non-encapsulated oil underscore the potential of alginate encapsulation in improving the overall antimicrobial performance of the essential oil. The improved stability and sustained release provided by the alginate encapsulation likely contributed to this enhancement, providing a more effective delivery system for the bioactive components.

Table 4: Inhibition bacteria activity of PEO microencapsulation

Bacteria	Material	Diameter of zone inhibition (mm)
Staphylococcus epidermidis	Positive Control (Ciprofloxacin 5 µg)	33.2±0.08
	Negative Control (aquadest)	6.03±0.05
	PEO 100%	12.50±0.14
	F1	11.70±0.16
	F2	10.93±0.21
	F3	12.30±0.16
Pseudomonas aeruginosa	Positive Control (Ciprofloxacin 5 µg)	34.90±0.14
	Negative Control (aquadest)	6.03±0.05
	PEO 100%	6.03±0.17
	F1	6.07±0.21
	F2	6.10±0.16
	F3	7.60±0.24

Data were presented in mean±SD, n=3

CONCLUSION

This study revealed that the findings of this study demonstrates that the concentration of alginate used for encapsulation significantly influences the properties and antibacterial activity of PEO. Higher alginate concentrations resulted in improved encapsulation efficiency, particle size distribution, and ultimately leading to enhanced antibacterial activity. These results highlight the potential of alginate-based encapsulation as a strategy to enhance the functional properties of essential oils, particularly their antimicrobial activity. Further research could explore the application of alginate-encapsulated essential oils in various delivery systems, such as coatings for personal care products. Understanding the impact of encapsulation on the performance of essential oils could pave the way for innovative and effective utilization of these natural compounds in diverse applications.

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AUTHORS CONTRIBUTIONS

Nastiti Utami contributed to the design and implementation of the study, the analysis of the results, and the writing of the manuscript. Retnaningtyas Kusuma Dewi conducted the experiments, formulated preparations, and tested the physical quality of the preparations. Dian Puspitasari supervised the formula. Novena Yety Lindawati analyzed the data. All authors discussed the results and commented on the manuscript.

CONFLICT OF INTERESTS

All the authors of this article declared no conflict of interest

REFERENCES

1 Utami N, Yety Lindawati N, Karunia Kristy T. Quality evaluation and effectiveness of palmarosa (*Cymbopogon martinii* var. motia) essential oils as repellents against Aedes aegypti. Res J Pharm Technol. 2023 Dec 29;16(12):5990-5. doi: 10.52711/0974-360X.2023.00972.

2 Prakash B, Kujur A, Yadav A, Kumar A, Singh PP, Dubey NK. Nanoencapsulation: an efficient technology to boost the antimicrobial potential of plant essential oils in food system. Food Control. 2018 Jul;89:1-11. doi: 10.1016/j.foodcont.2018.01.018.

3 Klosowska A, Wawrzynczak A, Feliczak Guzik A. Microencapsulation as a route for obtaining encapsulated flavors and fragrances. Cosmetics. 2023 Jan 29;10(1):26. doi: 10.3390/cosmetics10010026.

4 Sousa VI, Parente JF, Marques JF, Forte MA, Tavares CJ. Microencapsulation of essential oils: a review. Polymers. 2022 Apr 23;14(9):1730. doi: 10.3390/polym14091730, PMID 35566899.

5 Julaeha E, Nurzaman M, Wahyudi T, Nurjanah S, Permadi N, Anshori JA. The development of the antibacterial microcapsules of citrus essential oil for the cosmetotextile application: a review. Molecules. 2022 Nov 21;27(22):8090. doi: 10.3390/molecules27228090, PMID 36432192.

6 Enascuta CE, Stepan E, Oprescu EE, Radu A, Alexandrescu E, Stoica R. Microencapsulation of essential oils. Rev Chim. 2018 Aug 15;69(7):1612-5. doi: 10.37358/RC.18.7.6381.

7 Carvalho IT, Estevinho BN, Santos L. Application of microencapsulated essential oils in cosmetic and personal healthcare products a review. Int J Cosmet Sci. 2016;38(2):109-19. doi: 10.1111/ics.12232, PMID 25923295.

8 Froiio F, Ginot L, Paolino D, Lebaz N, Bentaher A, Fessi H. Essential oils loaded polymer particles: preparation characterization and antimicrobial property. Polymers. 2019 Jun 9;11(6):1017. doi: 10.3390/polym11061017, PMID 31181851.

9 Gayo CD. Pengaruh variasi konsentrasi natrium alginat terhadap efisiensi penjerapan mikrokapsul minyak biji jinten hitam (Nigella sativa L.); 2016.

10 Soliman EA, El Moghazy AY, El Din MS, Massoud MA. Microencapsulation of essential oils within alginate: formulation and *in vitro* evaluation of antifungal activity. J Encapsulation Adsorpt Sci. 2013 Jan 1;03(1):48-55. doi: 10.4236/jeas.2013.31006.

11 Wiegand I, Hilpert K, Hancock RE. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat Protoc. 2008;3(2):163-75. doi: 10.1038/nprot.2007.521, PMID 18274517.

12 Zorah M, Mudhafar M, Naser HA, Mustapa IR. The promises of the potential uses of polymer biomaterials in biomedical applications and their challenges. Int J App Pharm. 2023 Jul 7;15(4):27-36. doi: 10.22159/ijap.2023v15i4.48119.

13 Amatrejo S, HR T. Tinospora extract encapsulation with sodium alginate isolated from brown algae. Asian J Pharm Clin Res. 2017 Nov 1;10(11):267. doi: 10.22159/ajpcr.2017.v10i11.16517.

14 Samran S, Dalimunthe D, Dalimunthe D, Dalimunthe D. The formulation of dry curcuma extract microcapsules by spray wet microencapsulation techniques. Asian J Pharm Clin Res. 2018 Mar 1;11(3):226. doi: 10.22159/ajpcr.2018.v11i3.22608.

15 HU C, LU W, Mata A, Nishinari K, Fang Y. Ions induced gelation of alginate: mechanisms and applications. Int J Biol Macromol. 2021 Apr 30;177:578-88. doi: 10.1016/j.ijbiomac.2021.02.086, PMID 33617905.

16 Mergulhao NL, Bulhoes LC, Silva VC, Duarte IF, Basilio Junior ID, Freitas JD. Insights from Syzygium aromaticum essential oil: encapsulation characterization and antioxidant activity. Pharmaceuticals (Basel). 2024 May 8;17(5):599. doi: 10.3390/ph17050599, PMID 38794169.

17 Cimino C, Maurel OM, Musumeci T, Bonaccorso A, Drago F, Souto EM. Essential oils: pharmaceutical applications and encapsulation strategies into lipid based delivery systems. Pharmaceutics. 2021 Mar 3;13(3):327. doi: 10.3390/pharmaceutics13030327, PMID 33802570.

18 Suljagic J, Bratovcic A. Micro and nano-encapsulation in food industry. Croat J Food Sci Technol. 2019 May;11(1):113-21. doi: 10.17508/CJFST.2019.11.1.17.
19 Tan J, Luo Y, Guo Y, Zhou Y, Liao X, LI D. Development of alginate based hydrogels: crosslinking strategies and biomedical applications. Int J Biol Macromol. 2023 Jun;239:124275. doi: 10.1016/j.ijbiomac.2023.124275, PMID 37011751.
20 Abka Khajouei R, Tounsi L, Shahabi N, Patel AK, Abdelkafi S, Michaud P. Structures properties and applications of alginates. Mar Drugs. 2022 May 29;20(6):364. doi: 10.3390/md20060364, PMID 35736167.
21 MA D, Yang B, Zhao J, Yuan D, LI Q. Advances in protein based microcapsules and their applications: a review. Int J Biol Macromol. 2024 Jan;263(1):129742. doi: 10.1016/j.ijbiomac.2024.129742, PMID 38278389.
22 Jyothi NV, Prasanna PM, Sakarkar SN, Prabha KS, Ramaiah PS, Srawan GY. Microencapsulation techniques factors influencing encapsulation efficiency. J Microencapsul. 2010 May;27(3):187-97. doi: 10.3109/02652040903131301, PMID 20406093.
23 Sairam M, Babu VR, Vijaya B, Naidu K, Aminabhavi TM. Encapsulation efficiency and controlled release characteristics of crosslinked polyacrylamide particles. Int J Pharm. 2006;320(1-2):131-6. doi: 10.1016/j.ijpharm.2006.05.001, PMID 16766148.
24 Dolca C, Ferrandiz M, Capablanca L, Franco E, Mira E, Lopez F. Microencapsulation of rosemary essential oil by co-extrusion/gelling using alginate as a wall material. J Encapsulation Adsorpt Sci. 2015;05(3):121-30. doi: 10.4236/jeas.2015.53010.
25 Chouhan S, Sharma K, Guleria S. Antimicrobial activity of some essential oils present status and future perspectives. Medicines (Basel). 2017 Aug 8;4(3):58. doi: 10.3390/medicines4030058, PMID 28930272.
26 Rajagopal M, Walker S. Envelope structures of gram positive bacteria. Curr Top Microbiol Immunol. 2017;404:1-44. doi: 10.1007/82_2015_5021, PMID 26919863, PMCID PMC5002265.
27 Malanovic N, Lohner K. Gram positive bacterial cell envelopes: the impact on the activity of antimicrobial peptides. Biochim Biophys Acta. 2016;1858(5):936-46. doi: 10.1016/j.bbamem.2015.11.004, PMID 26577273.
28 Miller SI, Salama NR. The gram negative bacterial periplasm: size matters. PLOS Biol. 2018 Jan 17;16(1):e2004935. doi: 10.1371/journal.pbio.2004935, PMID 29342145, PMCID PMC5771553.
29 Rollauer SE, Sooreshjani MA, Noinaj N, Buchanan SK. Outer membrane protein biogenesis in gram negative bacteria. Philos Trans R Soc Lond B Biol Sci. 2015 Oct 5;370(1679):20150023. doi: 10.1098/rstb.2015.0023, PMID 26370935, PMCID PMC4632599.
30 Sun J, Rutherford ST, Silhavy TJ, Huang KC. Physical properties of the bacterial outer membrane. Nat Rev Microbiol. 2022 Apr;20(4):236-48. doi: 10.1038/s41579-021-00638-0, PMID 34732874, PMCID PMC8934262.
31 Rajagopal M, Walker S. Envelope structures of gram positive bacteria. Curr Top Microbiol Immunol. 2017;404:1-44. doi: 10.1007/82_2015_5021, PMID 26919863, PMCID PMC5002265.
32 Rajagopal M, Walker S. Envelope structures of gram positive bacteria. Curr Top Microbiol Immunol. 2017;404:1-44. doi: 10.1007/82_2015_5021, PMID 26919863, PMCID PMC5002265.
33 Gumbart JC, Beeby M, Jensen GJ, Roux B. Escherichia coli peptidoglycan structure and mechanics as predicted by atomic scale simulations. PLOS Comput Biol. 2014;10(2):e1003475. doi: 10.1371/journal.pcbi.1003475, PMID 24586129.
34 Barak I, Muchova K. The role of lipid domains in bacterial cell processes. Int J Mol Sci. 2013 Feb 18;14(2):4050-65. doi: 10.3390/ijms14024050, PMID 23429192, PMCID PMC3588084.
35 Pasquina Lemonche L, Burns J, Turner RD, Kumar S, Tank R, Mullin N. The architecture of the gram positive bacterial cell wall. Nature. 2020;582(7811):294-7. doi: 10.1038/s41586-020-2236-6, PMID 32523118.
36 Breijyeh Z, Jubeh B, Karaman R. Resistance of gram negative bacteria to current antibacterial agents and approaches to resolve it. Molecules. 2020 Mar 16;25(6):1340. doi: 10.3390/molecules25061340, PMID 32187986, PMCID PMC7144564.